Original Article

Role of P63 in Benign and Malignant Lesions of Breast

Jilla Rajitha[1], Biradar Rutushri Gangadhar[2], P. Indra Sekhar[3], Sumalatha Kasturi[4]

[1]Associate Professor, [2,3]Post Graduate, [4]Professor, Department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences, Bommakal, Karimnagar.

How to cite this article: Jilla Rajitha, Biradar Rutushri Gangadhar, P. Indra Sekhar et. al. Role of P63 in Benign and Malignant Lesions of Breast. Indian Journal of Public Health Research and Development/Volume 15 No. 2, April - June 2024.

Abstract

Introduction: Breast carcinoma is leading cause of cancer death in women. Breast lesions constitute heterogeneous group of diseases with wide variety of etiologies ranging from inflammatory-benign- malignant lesions. There are several reported markers for immunohistochemical detection of myoepithelial cells. Smooth muscle specific proteins, such as smooth muscle actin, smooth muscle myosin heavy chain, calponin and h-caldesmon are used to highlight myoepithelium. p63 antibody is myoepithelial cell marker that selectively stains nuclei. It is negative in stromal, myofibroblastic and adipocytic cells.

Aims and Objectives: The aim of this study was to establish role of p63 expression in distinguishing benign breast lesions, premalignant lesions and malignant tumors of breast.

Materials and Methods: 30 cases were selected from core biopsy, lumpectomy and mastectomy specimens of breast received at department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences during the period of January 2021 to December 2021 and were studied prospectively. All specimens were processed according to CAP protocol and reported . Immunohistochemistry was performed to determine p63 expression in those specimens. p63expression was evaluated as continuous positive/discontinuous positive/negative.

Ethical Approval: This study was reviewed and approved by institute ethics committee, CAIMS, Karimnagar.

Results: Among total 30 cases, 18 cases (60%) were benign lesions and all were positive for p63 expression. 3 cases (9.99%) were premalignant and were least positive for p63 expression. All malignant cases 9 cases (29.99%) were negative for p63 expression.

Conclusion: The Positive correlation was seen between histomorphological features and p63 scoring in all the lesions, So p63 is good Immunohistochemical marker for evaluating breast lesions.

Keywords: p63, Ductal carcinoma in situ, Fibroadenoma, Invasive ductal carcinoma, Myoepithelial cells.

Introduction

Breast lesions are heterogenous group of diseases having marked clinical and morphological diversity. [1] These are the most commonly associated lesions in women that require the prompt histopathological diagnosis and immunohistochemical analysis (IHC). [2] Breast cancer is the most common cancer in the world mainly in developed countries, it is the most

Corresponding Author: Jilla Rajitha, Associate Professor, Department of pathology, Chalmeda Anand Rao Institute of Medical Sciences, Bommakal, Karimnagar, Telangana, India.

E-mail: dr.rajitha25@gmail.com

Phone: 9849725682

Submission date: Jun 17, 2023, **Revision date:** Jun 22, 2023, **Published date: Apr 4 2024**

common cancer in women, breast cancers account for the 22% of all female cancers worldwide and 12% of breast cancer occurs in women between 20-34 years.[2,3] Ductal carcinomas arising from cells of the terminal ductal lobular units are reported to be the most common among all tumors.[4]

The layer of myoepithelial cells which lie between the luminal epithelial cells invests the glandular tree and is identifiable on routine hematoxylin and eosin (H&E) stained sections.[5] Immunohistochemical methods are being used to highlight the presence of intact myoepithelial cell layer.[6] The precise identification of myoepithelial cells can give diagnostic clue to differentiate benign lesions, benign proliferative lesions with similar morphological appearance, in situ neoplasms from invasive carcinoma of breast.[7]

Myoepithelial marker p63, a member of p53 gene family is expressed in the nuclei of myoepithelial cells of normal breast [6] and also expressed in epithelial cells of stratified epithelia such as skin, esophagus, ectocervix, transitional epithelia of bladder, basal cells of glandular structures of the prostate, salivary glands and in bronchi.[5,6]

p63 is more sensitive marker as it stains exclusively the nuclei of myoepithelial cells of the breast and do not cross react with stromal myofibroblasts, vascular smooth muscles and adipose tissue unlike other myoepithelial cell markers such as smooth muscle actin (SMA), calponin, caldesmon, smooth muscle myosin heavy chain (SMMHC), cytokeratins 5/6 and CD 10.[7,8] This makes p63 a more sensitive and the superior marker over other myoepithelial markers and can be included in IHC panels to identify the myoepithelial cells in problematic breast lesions. [9]

In normal breast, p63 is demonstrated as continuous intense staining pattern.[10] In the benign non-proliferative lesions it is continuously positive, in proliferative lesions it is discontinuously positive and in situ lesions show focal positivity.[11,12] Invasive carcinomas lack the myoepithelial cell layer and hence negative for p63 staining.[13,14] Thus p63 expression is of diagnostic clue to differentiate benign lesions, benign proliferative lesions with similar morpholological appearance, in situ neoplasms and invasive carcinoma of the breast.[15,16]

Materials and Methods:

Study design : Prospective

This study was studied on total of 30 cases of breast specimens received in the histopathology unit, Department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences, Bommakal, Karimnagar, irrespective of age and gender during the period from January 2021 to December 2021. Clinical history, informed consent and the examination findings of the patients were collected in all cases. All specimens were routinely processed and stained with hematoxylin and eosin (H&E) stain . The detailed histopathological examination (HPE) was done. Then the unstained sections were subjected to p63 antibody staining using Standard non-biotin polymerized horse radish peroxidase (HRP) technique to localise p63 antigen using the normal breast tissue as a positive control.

Inclusion criteria

- Patients of all age and both gender were included in the study.
- The Core needle biopsy, trucut biopsy of breast, lumpectomy and mastectomy specimens.

Exclusion criteria

- Inadequately fixed and processed specimens.

Table 1 : Distribution of different breast lesions

DIAGNOSIS	No of cases	Percentage (%)
BENIGN		
Fibroadenoma	9	30%
Fibrocystic disease	4	13.33%
Benign phyllodes tumor	1	3.33%
Tubular adenoma	1	3.33%
Usual ductal Hyperplasia	2	6.66%
Benign papilloma	1	3.33%
PREMALIGNANT		
Ductal carcinoma insitu	3	9.9%
MALIGNANT		
Infiltrating ductal carcinoma, not otherwise specified	7	23.33%
Papillary carcinoma	2	6.66%
TOTAL	30	100%

Immunohistochemical analysis/ scoring for breast lesions:

p63 expression was evaluated as continuous positive/ less continuous positive/ discontinuous positive/ Negative and scoring is done with reference to verma et.al.[10]

Results

Among total 30 cases in our study, 18 cases were benign which included 9 cases fibroadenoma (Figure 1A), 4 fibrocystic disease, 2 usual ductal hyperplasia, 1 benign phyllodes, 1 tubular adenoma and1 benign papilloma. Premalignant lesions include 3 cases of ductal carcinoma in situ (DCIS) and malignant cases include 7 cases of Infiltrating ductal carcinoma not otherwise specified (NOS) (Figure 1B) and 2 cases of papillary carcinoma (Table-1).

Age wise distribution

The age of patients ranged from 14 to 78 years and majority of cases 12 (40%) were between age group 35 - 50 years.(Table-2)

Table 2: Age wise distribution of cases

Age	<35 years	35-50 years	>50 years
No. Of cases	8	12	10

Size wise distribution

In our present study majority of cases i.e. 22 cases (73.33%) were of size 2-5 cm. The mean size of the benign tumor was 3.5 cm and malignant tumor was 7.5 cm. (Table-3).

Table 3 : Distribution of cases on the basis of size of lump (n=30)

Size (cm)	Benign	Malignant	Total
< 2cm	1(3.33%)	-	1(3.33%)
2-5 cm	16(53.33%)	5(16.66%)	21(69.99%)
> 5 cm	2(6.66%)	6(20%)	8(26.66%)
Total	19(63.33%)	11(36.66%)	30(100%)

P63 EXPRESSION:

Among total 30 cases, 18 cases (60%) were benign lesions and all were positive for p63 expression. 3 cases (9.99%) were premalignant and were least positive for p63 expression. All malignant cases 9 cases (29.99%) were negative for p63 expression. Among benign cases, the fibroadenoma was most common and showed the continuous p63 expression with score 3 (Figure-2A), Benign papilloma also showed continuous p63 expression with score 3. Other remaining benign lesions included in study like fibrocystic disease, Usual ductal hyperplasia and tubular adenoma (Figure- 2B) showed less continuous positive with score 2. Premalignant lesions like DCIS showed least positivity with score 1(Figure-3) and all the malignant lesions, invasive ductal carcinoma (Figure- 4) and the papillary carcinoma were negative for p63 expression.(Table-4)

Table 4 : Distribution of different breast lesions

DIAGNOSIS	No of cases	p63 scoring			
		Score-0	Score-1	Score-2	Score-3
BENIGN					
Fibroadenoma	9				+
Fibrocystic disease	4			+	
Benign phyllodes tumor	1		+		
Tubular adenoma	1			+	
Usual Ductal Hyperplasia	2			+	
Benign papilloma	1				+
PREMALIGNANT					
Ductal carcinoma insitu	3		+		
MALIGNANT					
Infiltrating ductal carcinoma, not otherwise specified	7	+			
Papillary carcinoma	2	+			
TOTAL	30				

Discussion

In our study, 60% cases were benign which is roughly close to the findings of Verma[1] et al with 67.6% and stefanaou [7]et.al with 52.63% and is higher than Werling [10] et. al who found percentage of benign cases to be 12.8% respectively.

In the present study, 29.99% cases were malignant which is slightly lower than the findings of Verma et al with 32.4% and much lower than Stefanaou et al[7] and Werling et al as their results were 36.09% and 41.17% respectively. Fibroadenoma accounted for 30% of all the breast lumps which was in agreement with most of the available literature on benign breast lumps, where the frequency ranged from 46.6%-67.6%. Invasive ductal carcinoma was the commonest malignant lesion in our study (23.33%), which was similar to findings of Verma et al with 27.5% cases of invasive ductal carcinoma and Stefanaou et al with 23.3% cases respectively.

Size And Age Distribution

In our study, all breast lumps were in range of 0.5-8cm and maximum of them (71.69%) had tumour size 2-5cm. Only 66 % of benign cases had tumor size more than 5cm while 20% of malignant cases had tumour size >5cm.

with a size >2cm and 10.9% with a size <2cm were malignant.

In our study, majority of cases 12 cases (40%) were in the age group of 35-50 years (39.62%) which was similar to study done by Verma et. Al[1], Stefanaou et.al[7] and Werling et.al[10]. Reibero-A[12] et.al have taken only the breast carcinoma cases in their study and the common age group in majority of cases was between 50-70 years.

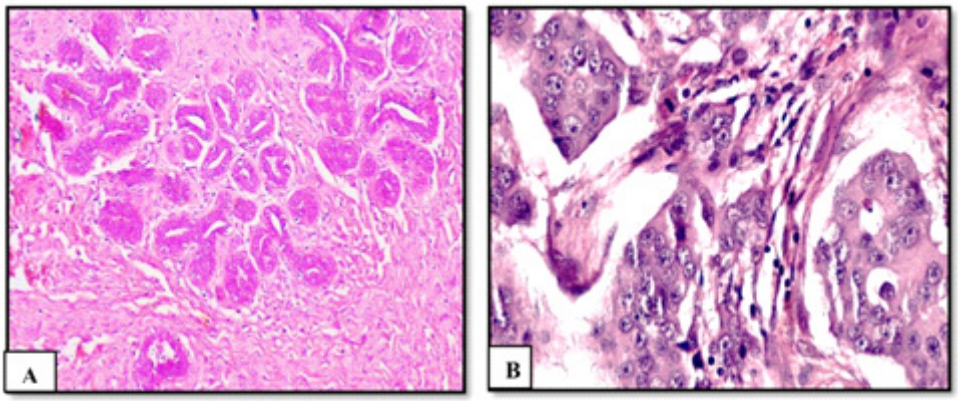

Figure-1: Microphotograph of : A) Fibroadenoma (H&E, 200x); B) Invasive papillary carcinoma (H&E, 400x)

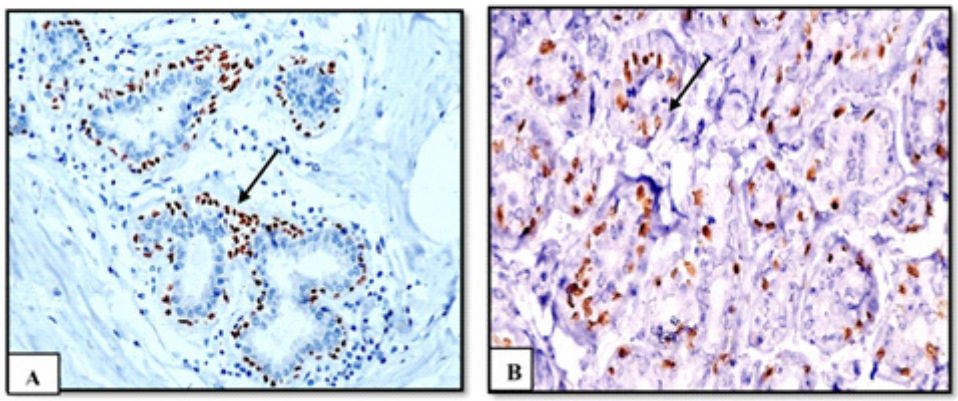

Figure-2: IHC: A- p63 expression in Fibroadenoma (Score-3) (200x)

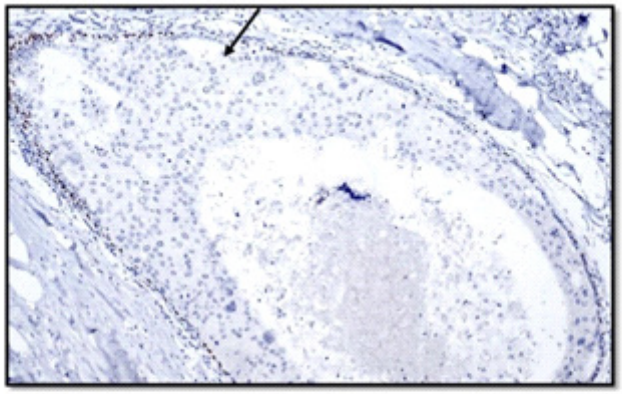

Figure-3: IHC - p63 expression in Ductal carcinoma in situ (Score – 1) (200x)

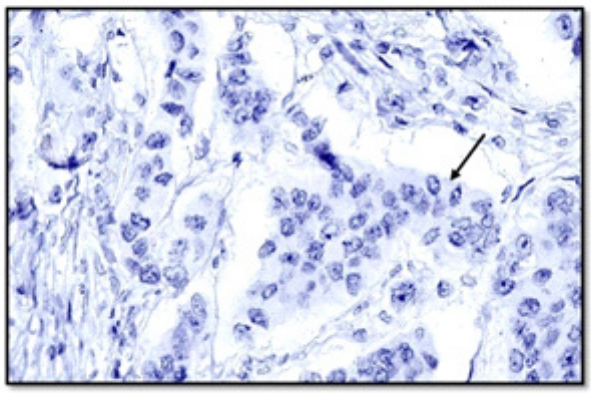

Figure-4: IHC - p63 expression in Invasive ductal carcinoma (NOS) (Score – 0) (400x)

P63 EXPRESSION

In our study, all the benign and premalignant tumors were positive for the p63 expression while 100% of the malignant tumors were devoid of p63 positivity. In 2000, Barbareschi M et al [5], investigated the 384 samples of normal and diseased human breast, including 300 invasive carcinomas, noted that p63 positivity was present in all benign lesions while invasive breast carcinomas were consistently devoid of the nuclear p63 staining. In 2002, Xiaojuan Wang[6] et al investigated 40 cases, all of which contained normal breast tissue, ductal hyperplasia, ductal carcinoma in situ and invasive ductal carcinoma; p63 was exclusively expressed in the myoepithelial cells of normal breast, partially expressed in ductal hyperplasia, rarely expressed in carcinoma in situ and not expressed in invasive carcinomas.

Conclusion

The pattern of p63 expression was studied on total of 30 cases in our study. The Positive correlation was seen between histomorphological features and p63 scoring in all the lesions. Majority of the cases were presented in age group of 35-50 yrs. Overall mean size of the tumor was 4. There was No correlation seen between age of patient, size of lesions, lymph node status, histologic grading and staging with the p63 expression in our study. Among the benign category, the non-proliferative lesions were continuous positive, the proliferative lesions showed less continuous positivity for p63, premalignant lesions showed least positivity and all the malignant lesions were devoid of p63 staining.Thus our study suggests that p63 expression has helped us to find the existence of myoepithelial cells in breast lesions as well as its pattern of expression has helped us in differentiating many complex epithelial lesions of the breast; suggesting p63 is good immunohistochemical marker for evaluating breast lesions.

Conflict of Interest: NIL

Funding : NIL

References

1. Verma N, Sharma B, Singh P, Sharma SP, Rathi M, Raj D. Role of p63 expression in non- proliferative and proliferative lesions of breast. Int J Res Med Sci.2018;6(8):2705-10.
2. Silva AR, Ramalho LNZ, Garcia SB, Zucoloto S. Is p63 reliable in detecting microinvasion in ductal carcinoma in situ of the breast? Pathol Onchol Res. 2003;9(1): 20-3.
3. Khazai L, Rosa M. Use of immunohistochemical stains in epithelial lesions of the breast. Cancer control.2015;22(2):220-5.
4. Corben AD, Lerwill MF. Use of myoepithelial cell markers in the differential diagnosis of benign, insitu and invasive lesions of the breast. Int J Surg Pathol. 2009:351-73.
5. Barbareschi M, Pecciarini L, Cangi MG et al. p63, a p53 homologue, is a selective nuclear marker of myoepithelial cells of the human breast. Am J Surg Pathol.2001;25(8):1054-60.
6. Wang et al. p63 expression in normal, hyperplastic and malignant breast tiudsssue. Breast cancer. 2002;9(3):216-219.
7. Stefanou D, Batistatou A, Nonni A, Arkoumani E, Agnantis NJ . p63 expression in benign and malignant breast lesions. Histol Histopathol .2004;19:465-71.
8. Zaha DC. Significance of immunohistochemistry in breast cancer. World J Clin Onchol.2014;5(3):382-92.
9. Abdallah DM, MF EI Deeb N. Comparative immunohistochemical study of p63, SMA, CD10 and calponin in distinguishing in situ from invasive breast carcinoma. J Mol Biomark Diaagn. 2017;8(4): 2155-9929. Bibliography Page 91
10. Werling RW, Hwang H, Yaziji H, Gown AM. Immunohistochemical distinction of invasive from non-invasive breast lesions. A comparative study of p63 versus calponin and smooth muscle myosin heavy chain. Am J SurgPathol.2003;27(1):829.
11. Koker MM, Kleer CG. p63 expression in breast cancer – A highly sensitive and specific marker of metaplastic carcinoma. Am J Surg Pathol.200428(11):1506-12.
12. Ribeiro A, Ramalho LN, Garcia SB, Brandao DF, Chahud F, Zucoloto S. p63 correlates with both BRCA1 and cytokeratin 5 in invasive breast carcinomas: further evidence for the pathogenesis of the basal phenotype of breast cancer. Histopathology. 2005;47(5):458-66.
13. Silva AR, Ramalho LNZ, Garcia SB, Zucoloto S. The relationship between p63 and p53 expression in normal and neoplastic breast tissue. Arch Pathol Lab Med. 2003;127: 336-40.
14. Reisenbichler ES, Ross JR, Hameed O. The clinical use of aP63/cytokeratin7/18/cytokeratin5/14 antibody cocktail in diagnostic breast pathology.Ann Diag Pathol.2014; 18(6): 313-8.
15. Moraes schenka NG, Schenka AA, De Souza Queiroz L, De Almeida Matsura M, Vassallo J, Alvarenga M. Use of p63 and CD10 in the differential diagnosis of papillary neoplasms of the breast. Breast J. 2008;14(1):68-75.
16. Di Como et al. p63 expression in normal and tumor tissues.2002.Clin. Cancer Res.2002;8:494-501.
17. Kaufmann O, Fietze E, Mengs J. Value of p63 and cytokeratin 5/6 as immunohistochemical markers for the differential diagnosis of poorly differentiated and undifferentiated carcinomas. Am L Clin Pathol. 2009;116:823- 30.
18. Russell et al. Myoepithelial cell differentiation markers in ductal carcinoma in situ progression. Am J Pathol.2015;185:3076-89.
19. Moriya T, Kozuka Y, Kanomata n, Tse GM, Tan PH. The role of immunohistochemistry in the differential diagnosis of breast lesions. Pathology. 2009;41(1):68-71.
20. Gottlieb C, Raju U, Greenwald KA. Myoepithelial cells in the differential diagnosis of complex benign and malignant breast lesions: an immunohistochemical study. Mod Pathol.2013;3:135-40.
21. Liu H. application of immunohistochemistry in breast pathology. A review and update.2014;138:1629-42.
22. Zhao L, Yang X, khan A. Diagnostic role of immunohistochemistry in the evaluation of breast pathology specimens. Arch Pathol Lab Med.2014;138(1):16- 24.
23. Shekhar MP, Kato I, Nangia-Makker P, Tait L. Comedo-DCIS is a precursor lesion for basal-like breast carcinoma: identification of a novel p63/ Her2/neu expressing subgroup. Oncotarget. 2013 Feb;4(2):231-41.
24. Gusterson BA et al. Distribution of myoepithelial cells and basement membrane proteins in the normal breast and in benign and malignant breast diseases. Cancer Res. 2012;42:4763-4770.
25. Shamloula MM, Shorbagy SH, Saied EME. p63 and cytokeratin 8/18 expression in breast atypical ductal hyperplasia, ductal carcinoma in situ and invasive duct carcinoma.Journal of Egyptian Nat. Cancer Inst. 2007;19(3):202-210.